



DECLARATION

I, Koji YOKOKAWA—of 7-13, Nishi-Shimbashi 1-chome, Minato-ku, Tokyo 105-8408 Japan—hereby declare that I am conversant in both Japanese and English and that I believe the following is a true and correct translation of Japanese Patent Application No. 2003-026406.

Date: August 2, 2007

A handwritten signature in black ink, consisting of several fluid, overlapping loops and strokes, positioned above a horizontal line.

Koji YOKOKAWA



PATENT OFFICE
JAPANESE GOVERNMENT

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the following application as filed with this office.

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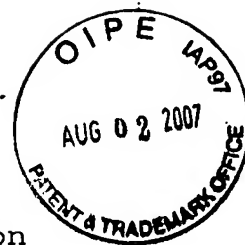
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[Article name]	Abstract	1
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[Necessity of Proof] Yes

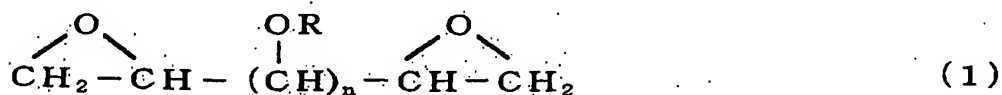


[Designation of Document] Specification

[Title of the Invention] Hyperbranched polymers derived from anhydrosugar-related compounds and process for the preparation thereof

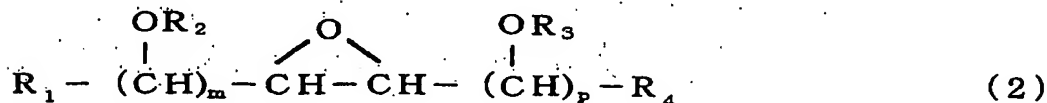
[Claims]

[Claim 1] A hyperbranched polymer comprising at least one sugar compound selected from a dianhydrosugar alcohol represented by the following general formula [1]:



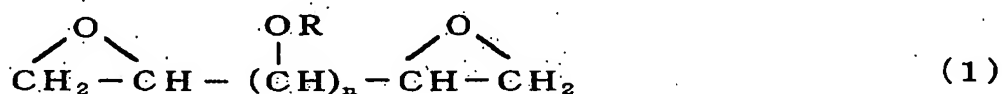
(wherein R's are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms, provided that at least one R of nR's is a hydrogen atom, and symbol n is an integer from 1 to 10).

[Claim 2] A hyperbranched polymer comprising at least one sugar compound selected from an anhydrosugar alcohol represented by the following general formula [2]:

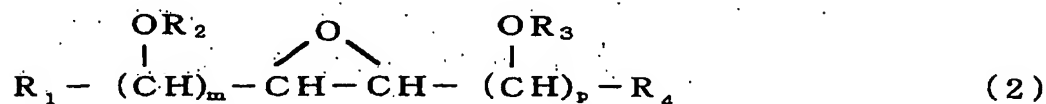


(wherein R_1 , R_2 's, R_3 's and R_4 are the same or different and each are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms, provided that at least one R_2 or R_3 of said mR_2 's and pR_3 's is a hydrogen atom; and symbol m is zero (0) or an integer from 1 to 20 and symbol p is an integer from 1 to 20, provided that symbol $m+p$ is an integer from 1 to 20).

[Claim 3] A hyperbranched polymer comprising a mixture of at least one sugar compound selected from a dianhydrosugar alcohol represented by the following general formula [1]:



(wherein R 's are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms, provided that at least one R of nR 's is a hydrogen atom, and symbol n is an integer from 1 to 10), and at least one sugar compound selected from an anhydrosugar alcohol represented by the following general formula [2]:

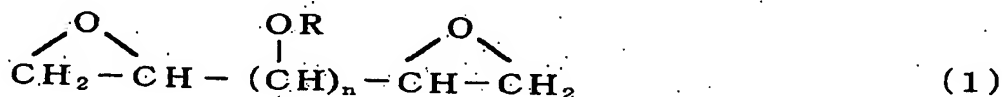


(wherein R_1 , R_2 's, R_3 's and R_4 are the same or different and each are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms, provided that at least one R_2 or R_3 of said mR_2 's and pR_3 's is a hydrogen atom; and symbol m is zero (0) or an integer from 1 to 20 and symbol p is an integer from 1 to 20, provided that symbol $m+p$ is an integer from 1 to 20).

[Claim 4] The hyperbranched polymer as claimed in any one of claims 1 to 3, wherein said hydrocarbon group is an alkyl group, an aryl group or an arylalkyl group.

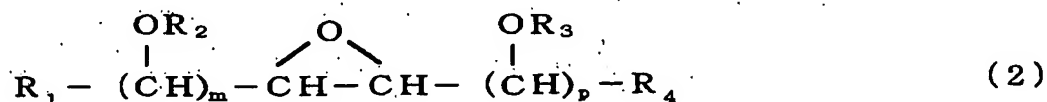
[Claim 5] The hyperbranched polymer as claimed in any one of claims 1 to 4, wherein a degree of branching is from 0.05 to 1.00.

[Claim 6] A process for the preparation of a hyperbranched polymer comprising polymerizing at least one sugar compound selected from a dianhydrosugar alcohol represented by the following general formula [1]:



(wherein R's are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms, provided that at least one of nR's is a hydrogen atom, and symbol n is an integer from 1 to 10) in the presence of a cationic initiator or an anionic initiator.

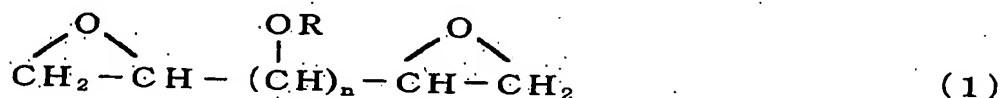
[Claim 7] A process for the preparation of a hyperbranched polymer comprising polymerizing at least one sugar compound selected from an anhydrosugar alcohol represented by the following general formula [2]:



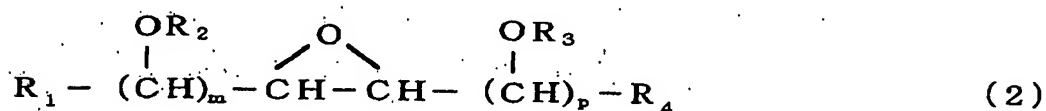
(wherein R₁, R₂'s, R₃'s and R₄ are the same or different and each are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms, provided that at least one R₂ or R₃ of said mR₂'s and pR₃'s is a hydrogen atom; and symbol m is zero (0) or an integer from 1 to 20 and symbol p is an integer from 1 to 20, provided that symbol m+p is an integer from 1 to 20) in the presence of a cationic initiator or an anionic initiator.

[Claim 8] A process for the preparation of a hyperbranched polymer comprising polymerizing a mixture of at least one

anhydrosugar compound selected from a dianhydrosugar alcohol represented by the following general formula [1]:



(wherein R's are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms, provided that at least one of nR's is a hydrogen atom, and symbol n is an integer from 1 to 10), and at least one sugar compound selected from an anhydrosugar alcohol represented by the following general formula [2]:



(wherein R₁, R₂'s, R₃'s and R₄ are the same or different and each are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms, provided that at least one R₂ or R₃ of said mR₂'s and pR₃'s is a hydrogen atom; and symbol m is zero (0) or an integer from 1 to 20 and symbol p is an integer from 1 to 20, provided that symbol m+p is an integer from 1 to 20) in the presence of a cationic initiator or an anionic initiator.

[Claim 9] The method for the preparation of the hyperbranched polymer as claimed in any one of claims 6 to 8, wherein said hydrocarbon group is an alkyl group, an aryl group or an arylalkyl group.

[Claim 10] The method for the preparation of the hyperbranched polymer as claimed in any one of claims 6 to 9, wherein a degree of branching is from 0.05 to 1.00.

[Detailed Description of the Invention]

[Technical Field to which the Invention Belongs]

The present invention relates to anhydrosugar-related hyperbranched polymers useful for a viscosity increasing agent as a biocompatible hydrogel or for a medical material such as an artificial coating agent; and a process for producing the same.

[Related Art]

A known method for obtaining a hyperbranched carbohydrate polymer includes, for example, the polymerization by the cationic ring cleavage of 1,6-anhydro-.beta.-D-glucopyranose [e.g., Schuerch et al.: J. Am. Chem. Soc., vol. 81, p. 4054, 1959 (non-patent document 1)].

This method, however, suffers from the difficulty in controlling a degree of branching and a molecular weight. The resulting hyperbranched carbohydrate polymer is restricted to a sugar having a glucoside linkage composed of a naturally occurring pyranosyl ring. Further, it is difficult to make up a versatile design of hyperbranched carbohydrate polymers including other sugars consisting of furanosyl ring for example or other sugars having no glucoside linkage.

Recently, the synthesis of hyperbranched polyaminosugars by the glycosylation reaction of sugar oxazoline derivatives are reported [Kadokawa, et al.: Angew. Chem. Int. Ed., vol. 37, pp. 2373-2376, 1998 (non-patent document 2); Polym. Adv. Technol., vol. 11, p. 122, 2000 (non-patent document 3)]. This process is solely applicable to aminosugars and it has the difficulty of the application to other sugars.

non-patent document 1 : Schuerch et al.: J. Am. Chem. Soc., vol. 81, p. 4054, 1959 (non-patent document 1)

non-patent document 2 : Kadokawa, et al.: Angew. Chem. Int. Ed., vol. 37, pp. 2373-2376, 1998

non-patent document 3 : Polym. Adv. Technol., vol. 11, p. 122, 2000

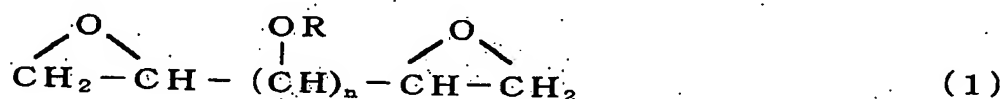
[Problems that the Invention is to Solve]

The object of the present invention is to provide hyperbranched polymers derived from anhydrosugar-related compounds which can be produced easily; and a process for producing the same.

[Means for Solving the Problems]

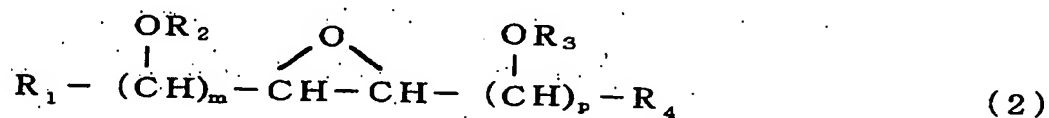
According to the present invention, the following hyperbranched polymers and the process for producing the same are provided.

(1) A hyperbranched polymer comprising at least one sugar compound selected from a dianhydrosugar alcohol represented by the following general formula [1]:



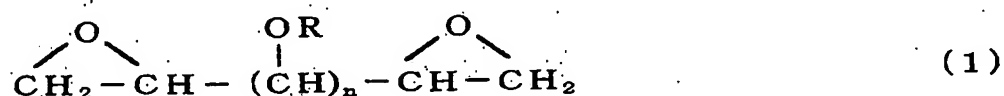
(wherein R's are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms, provided that at least one of nR's is a hydrogen atom, and symbol n is an integer from 1 to 10).

(2) A hyperbranched polymer comprising at least one sugar compound selected from an anhydrosugar alcohol represented by the following general formula [2]:

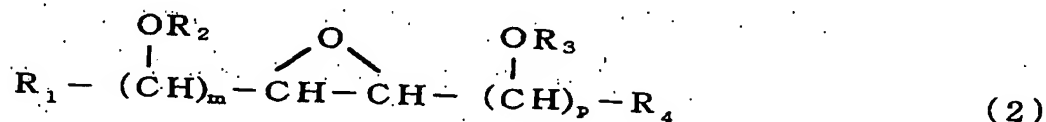


(wherein R_1 , R_2 's, R_3 's and R_4 are the same or different and each are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms, provided that at least one R_2 or R_3 of said mR_2 's and pR_3 's is a hydrogen atom; and symbol m is zero (0) or an integer from 1 to 20 and symbol p is an integer from 1 to 20, provided that symbol $m+p$ is an integer from 1 to 20).

(3) A hyperbranched polymer comprising a mixture of at least one sugar compound selected from a dianhydrosugar alcohol represented by the following general formula [1]:



(wherein R 's are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms, provided that at least one of nR 's is a hydrogen atom, and symbol n is an integer from 1 to 10), and at least one sugar compound selected from an anhydrosugar alcohol represented by the following general formula [2]:

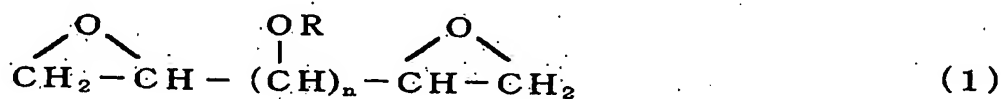


(wherein R_1 , R_2 's, R_3 's and R_4 are the same or different and each are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms, provided that at least one R_2 or R_3 of said mR_2 's and pR_3 's is a hydrogen atom; and symbol m is zero (0) or an integer from 1 to 20 and symbol p is an integer from 1 to 20, provided that symbol $m+p$ is an integer from 1 to 20).

(4) The hyperbranched polymer as claimed in any one of (1) to (3), wherein said hydrocarbon group is an alkyl group, an aryl group or an arylalkyl group.

(5) The hyperbranched polymer as claimed in any one of (1) to (4), wherein a degree of branching is from 0.05 to 1.00.

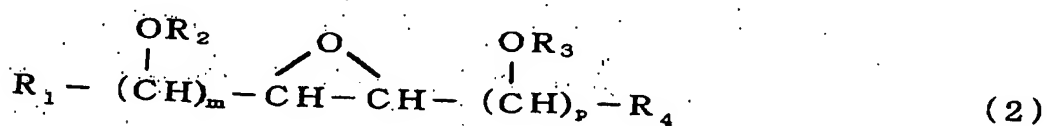
(6) A process for the preparation of a hyperbranched polymer comprising polymerizing at least one sugar compound selected from a dianhydrosugar alcohol represented by the following general formula [1]:



(wherein R 's are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms, provided that at least one of nR 's is a hydrogen atom, and symbol n is an integer

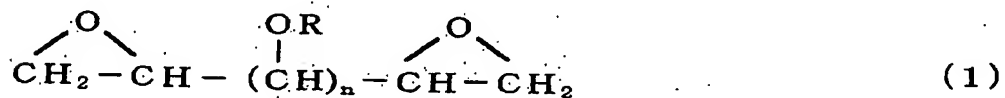
from 1 to 10) in the presence of a cationic initiator or an anionic initiator.

(7) A process for the preparation of a hyperbranched polymer comprising polymerizing at least one sugar compound selected from an anhydrosugar alcohol represented by the following general formula [2]:

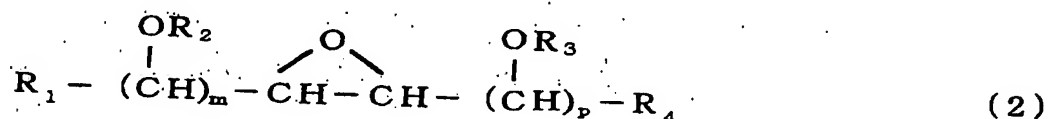


(wherein R_1 , R_2 's, R_3 's and R_4 are the same or different and each are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms, provided that at least one R_2 or R_3 of said mR_2 's and pR_3 's is a hydrogen atom; and symbol m is zero (0) or an integer from 1 to 20 and symbol p is an integer from 1 to 20, provided that symbol $m+p$ is an integer from 1 to 20) in the presence of a cationic initiator or an anionic initiator.

(8) A process for the preparation of a hyperbranched polymer comprising polymerizing a mixture of at least one sugar compound selected from a dianhydrosugar alcohol represented by the following general formula [1]:



(wherein R's are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms, provided that at least one of nR's is a hydrogen atom, and symbol n is an integer from 1 to 10), and at least one sugar compound selected from an anhydrosugar alcohol represented by the following general formula [2]:



(wherein R₁, R₂'s, R₃'s and R₄ are the same or different and each are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms, provided that at least one R₂ or R₃ of said mR₂'s and pR₃'s is a hydrogen atom; and symbol m is zero (0) or an integer from 1 to 20 and symbol p is an integer from 1 to 20, provided that symbol m+p is an integer from 1 to 20) in the presence of a cationic initiator or an anionic initiator.

(9) The method for the preparation of the hyperbranched polymer as claimed in any one of (6) to (8), wherein said hydrocarbon group is an alkyl group, an aryl group or an arylalkyl group.

(10) The method for the preparation of the hyperbranched polymer as claimed in any one of (6) to (9), wherein a degree of branching is from 0.05 to 1.00.

[Best Mode for Carrying Out the Invention]

In the general formula [1], R's represent a hydrogen atom or a hydrocarbon group having 1 to 30 carbon atoms, preferably 1 to 10 carbon atoms. The hydrocarbon group as used herein may include a linear hydrocarbon group or a cyclic hydrocarbon group. The linear hydrocarbon group may include, for example, a linear hydrocarbon group derived from an aliphatic hydrocarbon, such as an alkyl group having from 1 to 30 carbon atoms, preferably from 1 to 4 carbon atoms, and an alkenyl group having from 2 to 30 carbon atoms, preferably from 2 to 5 carbon atoms. The alicyclic hydrocarbon group may include, for example, a cycloalkyl group or a cycloalkenyl group, each having from 3 to 30 carbon atoms, preferably from 5 to 7 carbon atoms.

The aromatic hydrocarbon group may include an aryl group having from 6 to 30 carbon atoms, preferably from 6 to 12 carbon atoms, and an arylalkyl group having from 7 to 30 carbon atoms, preferably from 7 to 10 carbon atoms.

Among the hydrocarbon groups as defined herein, the hydrocarbon group selected from the alkyl group, the aryl group and the arylalkyl group is preferred.

In the general formula [1], the symbol n is an integer from 1 to 10 carbon atoms, preferably from 1 to 4.

In the general formula [1], at least one R of nR 's is a hydrogen atom that is involved in the polymerization of the dianhydrosugar alcohol and assists in increasing a degree of branching for the resulting hyperbranched polymer (hyperbranched polysaccharide).

Examples of the dianhydrosugar alcohols represented by the general formula [1] may include a 1,2-5,6-dianhydro-D-mannitol-type compound, a 1,2-5,6-dianhydro-L-iditol-type compound, a 1,2-5,6-dianhydro-annitol-type compound, a 1,2-5,6-dianhydro-galactitol-type compound, a 1,2-5,6-dianhydro-glucitol-type compound, a 1,2-4,5-dianhydro-xylitol-type compound, and the like.

In the general formula [2], R_1 to R_4 each are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms.

In this case, the hydrocarbon group may include those shown above with regard to the general formula [1]. The hydrocarbon group is preferably an alkyl group, an aryl group and an arylalkyl group.

In the general formula [2], the symbol m is zero (0) or an integer from 1 to 20, preferably zero (0) or an integer from 1 to 4, and, likewise, the symbol p is an integer from 1 to 20, preferably an integer from 1 to 4,

however, provided that the symbol $m+p$ is an integer from 1 to 20.

Further, at least one R_2 or R_3 of mR_2 's and p_3 's is a hydrogen atom that is involved in the polymerization of the anhydrosugar alcohol represented by the general formula [2] and assists in increasing the degree of branching of the resulting hyperbranched polymer (hyperbranched polyether).

Examples of the anhydrosugar alcohol represented by the general formula [2] may include a 1,2-anhydro-D-mannitol-type compound, a 1,2-anhydro-L-iditol-type compound, a 1,2-anhydro-annitol-type compound, a 1,2-anhydro-galactitol-type compound, a 1,2-anhydro-glucitol-type compound, a 1,2-anhydro-xylitol-type compound, a 1,2-anhydro-threitol-type compound, and the like.

The hyperbranched polymer of the present invention can be produced by polymerizing at least one sugar compound selected from the dianhydrosugar alcohols represented by the general formula [1] and the general formula [2] in the presence of the cationic or anionic initiator used as a polymerization initiator.

As the cationic initiator to be used for the process according to the present invention, there may be used any one customarily used. Examples thereof include a thermal cationic initiator and an photocationic initiator such as

sulfonium antimonate, a Lewis acid such as boron trifluoride diethyl etherate, tin tetrachloride, antimony pentachloride, phosphorus pentafluoride, a Brenstead acid such as trifluoromethane sulfonic acid.

As the anionic initiator, there may be used any one customarily used, and examples thereof include and not be limited to a hydroxide such as KOH, and a metal alcolate such as tert-BuOK, and $\text{Zn}(\text{OCH}_3)_2$.

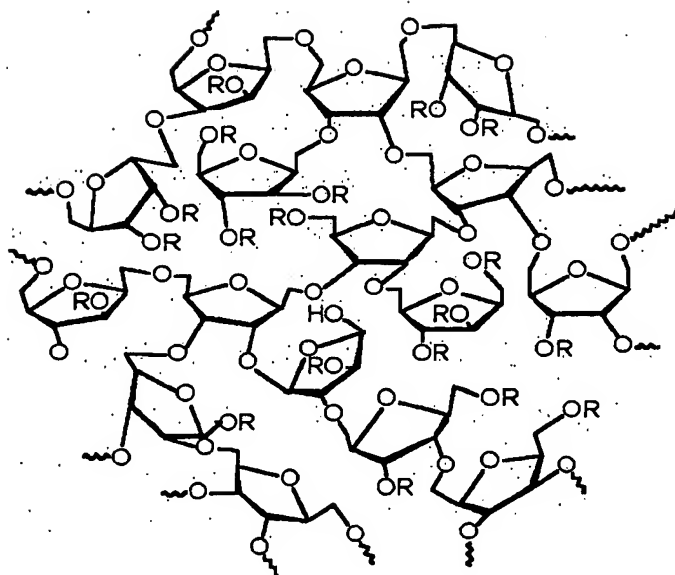
The amount of the initiators may be in the range of 1 to 10% by weight with respect to the anhydrosugar-related compound as a starting material.

The polymerization reaction to be used for the method of the present invention may be carried out in the presence of an organic solvent, such as tetrahydrofuran, dichloromethane and propylene carbonate.

The polymerization of the dianhydrosugar alcohol represented by the general formula [1] according to the method of the present invention will be described as a reference to the mechanism of the polymerization. The polymerization of the dianhydrosugar alcohol represented by the general formula [1] to be used as a starting material may involve the ring-opening and the ring-forming by the nucleophillic or electrophillic reaction. The reaction of an anhydro group with a hydroxyl group in other anhydrosugar can form a hyperbranched carbohydrate

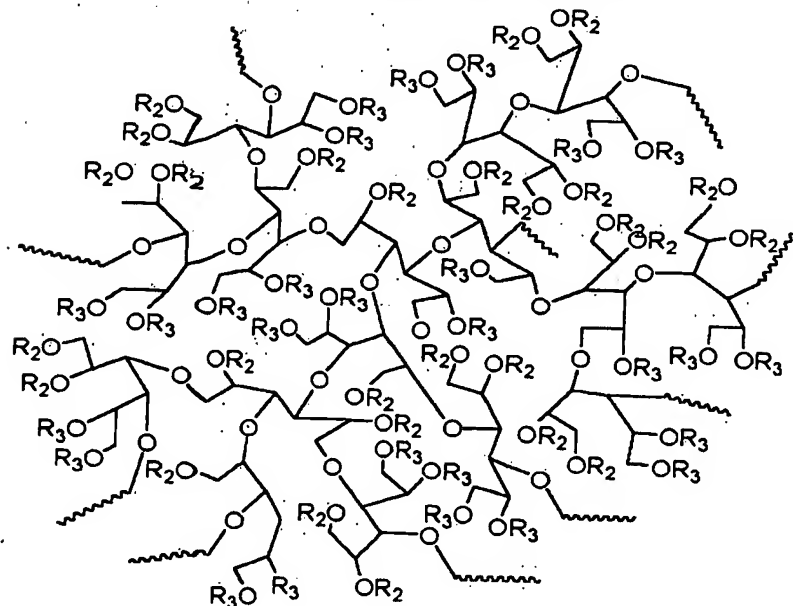
polymer consisting of a recurring unit without glycoside linkage, which does not occur naturally. In the case where the anhydrosugar alcohol as represented by the general formula [2] is used, the anhydro groups at the 1,2-, 1,4-, 1,5-, 2,3- or 3,5-positions may be cleaved by the hydroxyl group of the other sugar, thereby forming a highly branched polyether structure, that is, hyperbranched polyether. Further, when the dianhydrosugar alcohol represented by the general formula [1] is admixed with the anhydrosugar alcohol represented by the general formula [2], the polymerization reaction of the anhydrosugar alcohols of the general formulas [1] and [2] are allowed to occur simultaneously yielding a polymer that contains both of the hyperbranched carbohydrate and the hyperbranched polyether structure.

The chemical structure of the dianhydrosugar alcohol ($n=2$) of the general formula [1] may be represented by the general formula (3):



(3)

The chemical structure of the anhydrosugar alcohol ($R_1=R_4=H$, $m=p=2$) of the general formula [2] may be represented by the general formula (4):



(4)

The hyperbranched polymer according to the present invention has the degree of branching in the range of 0.05

to 1.00, preferably in the range of 0.45 to 1.0, when measured by the following Frechet's formula:

Degree of branching=(number of branched units+number of polymer terminals)/(number of branched units+number of polymer terminals+number of linear units).

The Frechet's formula can give the degree of branching of zero (0) for a linear polymer and the degree of branching of one (1) for a dendrimer.

The hyperbranched polymer according to the present invention may be in a shape close to a dendritic form and have further branches from the branched chain. The hyperbranched polymer according to the present invention may include a carbohydrate polymer composed of unique and non-naturally occurring recurring units having no glycoside linkage, that is, a fructose-type sugar unit.

The hyperbranched polymer comprising the dianhydrosugar alcohols of the general formula [1] and/or the anhydrosugar alcohols of the general formula [2] may have a molecular weight in the range of normally 10,000 or more, preferably 200,000 or more, when measured by the static laser light scattering method.

[Effect of the Invention]

The present invention can provide the synthesis of a water-soluble hyperbranched polymer with high reproductivity and on a large scale, allowing the use of the hyperbranched polymer as a functional material on an industrial scale. Moreover, the present invention can produce a hyperbranched carbohydrate polymer having a structure that cannot be obtained from a natural branched carbohydrate polymer, such as amylopectin or the like, and further provide the hyperbranched carbohydrate and/or polyether or mix of both structures that can be controlled a molecular weight and a degree of branching so as to comply with usage.

Further, the hyperbranched polymer according to the present invention can be applicable to medical materials such as a biocompatible hydrogel for a viscosity increasing agent or an artificial coating agent, and the like.

[Examples]

The present invention will be described in more detail by way of examples.

EXAMPLE 1

Cationic Polymerization of 1,2-5,6-dianhydro-D-mannitol

To a solution of 1,2-5,6-dianhydro-D-mannitol (0.50 g) in dry dichloromethane (6.8 mL, manufactured by Kanto Chemical Co., Inc., monomer concentration: $0.5 \text{ mol} \cdot \text{L}^{-1}$) was added boron trifluoride diethyl etherate (2.6 μL , manufactured by Kanto Chemical Co., Inc.) as a polymerization initiator at 0°C to start polymerization. After 200 hours, the reaction mixture was poured into a large amount of methanol and neutralized with aqueous ammonia. The solvent was evaporated, and the residue was purified using a permeable membrane in the water to give the polymer 0.209 g (41.8% yield). The weight average molecular weight (SEC, at 40°C in aqueous sodium nitrate solution (0.2 mol/L)) was 1,900 and the dispersion degree was 2.23. The weight average molecular weight (SLS, 0.2 sodium nitrate solution, 40°C) was 3.1×10^5 in aqueous sodium nitrate solution (0.2 mol/L). The inertial and hydrodynamic radii of the polymer were 83 nm (SLS) and 67 nm (DLS), respectively, at 40°C in aqueous sodium nitrate solution (0.2 mol/L).

The solubility of the product (hyperbranched sugar chain) in various solvents (concentration: 30 mg/mL, solution time: 1 hour) are shown in Table 1.

Table 1

H ₂ O	MeOH	acetone	CH ₂ Cl ₂	CHCl ₃	THF	toluene	pyridine
○	○	○	×	×	×	×	×

In Table 1, "○" means "soluble", and "×" means "not soluble".

The ¹H NMR and ¹³C NMR spectra of the polymer are shown in FIG. 1 and FIG. 2, respectively. For the linear carbohydrate polymers, a peak derived from the C1 carbon terminal was little demonstrated, while a large peak (nearby 60 ppm) derived from the C1 carbon terminal of the carbohydrate polymer according to the present invention was demonstrated. Moreover, the peak derived from the other carbon is divided into several peaks so that it is found to be in a hyperbranched structure. It was also found that the weight-average molecular weight measured by the SEC was demonstrated smaller than the weight-average molecular weight measured by the static laser light scattering method. This tendency seems to be based on the difference of the effective volume of the polymer and it can often be observed in the hyperbranched polymer.

EXAMPLE 2

Anionic Polymerization of 1,2-5,6-anhydro-D-mannitol

To a solution of 1,2-5,6-dianhydro-D-mannitol (0.50 g, manufactured from D-mannitol) in dry THF (3.4 mL,

manufactured by Kanto Chemical Co., Inc., monomer concentration: $1.0 \text{ mol} \cdot \text{L}^{-1}$) was added t-BuOK/THF solution (0.17 mL, manufactured by Kanto Chemical Co., Inc.) as a polymerization initiator under a nitrogen atmosphere to start polymerizaion. After 30 hours, the reaction mixture was poured into a large amount of methanol and neutralized with aqueous dry ice. The solvent was evaporated, and the residue was purified using a permeable membrane (Spectra pore M_w500) in water to give 0.085 g of the polymer. The yield was 17.0%; and a specific rotation (c 1.0, H_2O , 23°C). The weight average molecular weight (SEC, at 40°C in aqueous sodium nitrate solution (0.2 mol/L)) was 1,600 and the dispersion degree was 5.07. The weight average molecular weight (SLS, 0.2 sodium nitrate solution, 40°C) was 13.1×10^5 in aqueous sodium nitrate solution (0.2 mol/L). The inertial and hydrodynamic radii of the polymer were 131 nm (SLS) and 132 nm (DLS), respectively, at 40°C in aqueous sodium nitrate solution (0.2 mol/L). The ^1H NMR and ^{13}C NMR spectra of the polymer are shown in FIG. 3 and FIG. 4, respectively.

[Brief Description of the Drawings]

[FIG. 1] FIG. 1 is a diagram showing a spectrum of the hyperbranched carbohydrate polymer of Example 1 measured at 400 MHz and ^1H NMR (solvent: deuterium water, 25°C).

[FIG. 2] FIG. 2 is a diagram showing a spectrum of the hyperbranched carbohydrate polymer of Example 1 measured at 400 MHz and ^{13}C NMR (solvent: deuterium water, 25°C).

[FIG. 3] FIG. 3 is a diagram showing a spectrum of the hyperbranched carbohydrate polymer of Example 2 measured at 400 MHz and ^1H NMR (solvent: deuterium water, 25°C).

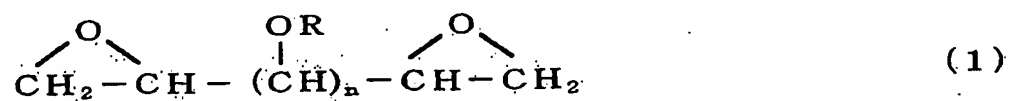
[FIG. 4] FIG. 4 is a diagram showing a spectrum of the hyperbranched carbohydrate polymer of Example 2 measured at 400 MHz and ^{13}C NMR (solvent: deuterium water, 25°C).

[Designation of Document] Abstract

[Abstract]

[Problem] To provide hyperbranched polymers derived from anhydrosugar-related compounds which can be produced easily.

[Means for Solving the Problem] A hyperbranched polymer comprising at least one sugar compound selected from a dianhydrosugar alcohol represented by the following general formula [1]:



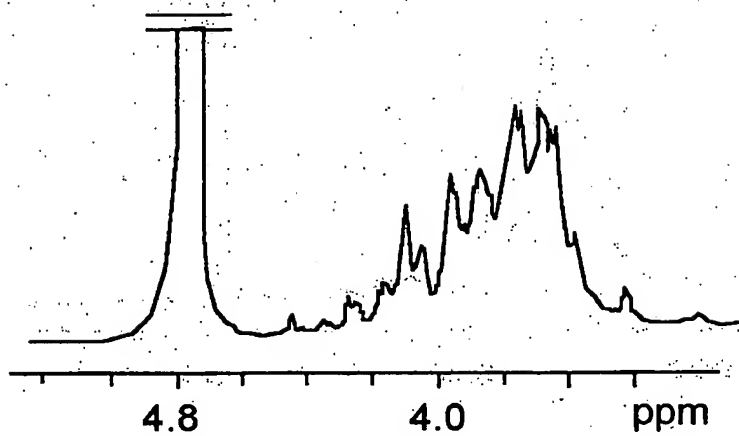
(wherein R's are a hydrogen atom or a hydrocarbon group having from 1 to 30 carbon atoms, provided that at least one R of nR's is a hydrogen atom, and symbol n is an integer from 1 to 10).

[Selected Drawing] None

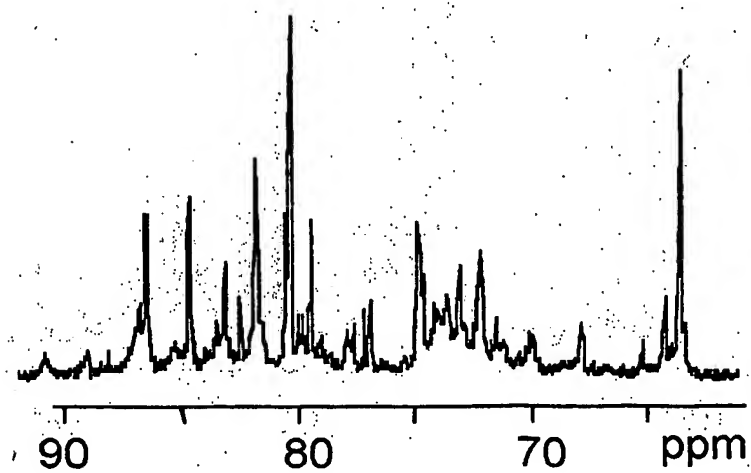


[Designation of Document] Drawings

[Fig. 1]

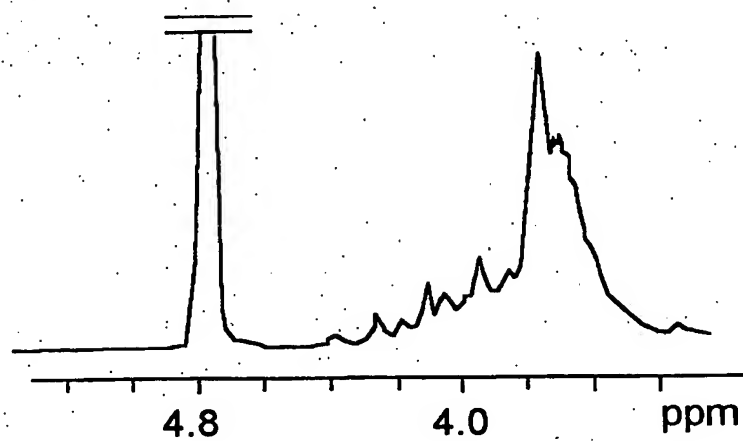


[Fig. 2]





[Fig. 3]



[Fig. 4]

